The effect of Dokudami (*Houttuynia cordata*) on expression of inflammatory related genes in human gingival epithelial cells stimulated by *Aggregatibacter actinomycetemcomitans*

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BACKGROUND

Periodontal disease is highly prevalent and can affect most of the world population. In Bangladesh, more than 80% of population is suffering from periodontal disease. Due to chronic periodontitis a big number of patients have been loosing their teeth. It has been reported the association between periodontal disease and many systemic diseases like diabetes, cardiovascular disease, stroke, pulmonary disease and adverse pregnancy outcomes. Therefore, it is necessary to investigate the preventive methods of periodontal disease.

Periodontitis is the inflammation of periodontal tissue that results from the interaction of plaque-associated bacteria with the host immune system. *Aggregatibacter actinomycetemcomitans* (*A.a*) is a gram-negative, facultative anaerobic coccobacillus that has the capacity to ferment many sugars and strongly implicated as a causitive organism in the early stage of periodontitis. Several virulence factors from *A. actinomycetemcomitans* have been identified including lipopolysaccharide, leukotoxin, cytolethal distending toxin, collagenase and outer membrane proteins. These virulence factors can induce the host inflammatory response.

The gingival epithelium is the primary barrier facing the bacterial challenge. Epithelial cells function as a mechanical barrier through cell-cell junctions complexes, for example, tight junctions and gap junctions, against invasion of pathogenic organism. Bacterial challenge to epithelial cells induces the production of inflammatory proteins such as interleukin (IL)-8, IL-6, matrixmetalloproteinase (MMP)-1 and-3, ICAM-1, TNF-α etc. Over production of these cytokines results in extension of inflammation and destruction of periodontal tissue.

Recently, natural products have been getting priority for the prevention of periodontal disease in the world. Dokudami (*Houttuynia cordata*) is the traditional oriental medicine, kampo medicine. It has been reported that dokudami has anti-inflammatory effects in various tissues. Natural products have a cost benefit on their production in developing country. Therefore, we think that dokudami is a candidate preventive medicine for periodontal disease. In this study, we examined the inhibitory effect of dokudami on the expression of inflammatory related genes in human gingival epithelial cells (HGEC) stimulated by *A. actinomycetemcomitans*.

MATERIALS AND METHODS:

HGEC has been used in this study. Human gingival tissues were collected from healthy
gingiva during the extraction of wisdom teeth under informed consent. HGEC were isolated. HGEC were pretreated with dokudami (0.5 and 5μg/ml), ERK inhibitors (PD98059), or p38 MAPK inhibitor (SB203580) for 30 min. After pretreatment, the cells were stimulated with A.a for 12h or 24h. Real time polymerase chain reaction and enzyme-linked immunosorbent assay were performed to examine IL-8 production at the mRNA and protein levels respectively. For Western blotting, after HGEC were pretreated with dokudami for 30 min, the cells were stimulated with A.a for 30 min. ERK phosphorylation were examined by Western blotting.

To reveal the effect of dokudami on inflammatory cytokine stimulation, we used TNF-α. After HGEC were pretreated with dokudami (0.5 and 5μg/ml) for 30 min, the cells were stimulated with TNF-α for 12h. Real time PCR was performed to examine IL-8, IL-6, ICAM-1, MMP-1 and MMP-3 mRNA expressions.

RESULTS :

A.a stimulation enhanced the IL-8 mRNA expression compared with control (without A.a stimulation) in HGEC. ELISA analysis showed the similar tendency in IL-8 proteins levels. However, dokudami suppressed the A.a-induced increase in IL-8 mRNA expression and protein production. A.a stimulation also induced the mRNA expression of IL-6, ICAM-1, MMP-1 and MMP-3 in HGEC, although dokudami also suppressed their expressions. ERK inhibitor pretreatment inhibited A.a-induced increase in IL-8 mRNA expression on HGEC. A.a stimulated the phosphorylation of ERK in HGEC. Dokudami suppressed A.a-induced phosphorylation of ERK. TNF-α stimulation enhanced the mRNA expression of IL-8, IL-6, ICAM-1, MMP-1 and MMP-3 compared with control in HGEC, although dokudami pretreatment suppressed these gene expressions.

DISCUSSION :

In this study, we demonstrated that natural product dokudami pretreatment was able to reduce the production on A.a-induced cytokines. We have investigated for the first time the suppressive effect of dokudami on inflammatory related genes expression in HGEC stimulated by A.a. Since previous study reported that the exposure of HGEC to A.a or IL-8 caused a decrease in gap junction intercellular communication in HGEC, dokudami might be control cell-cell junctional complex by regulating IL-8, MMP-1 and MMP-3. Thus, dokudami may control inflammatory response in HGEC induced by A.a. In this study, we also found dokudami suppressed IL-8 expression and phosphorylation of ERK in HGEC stimulated by A.a, suggesting that dokudami regulate IL-8 expression through ERK pathway.

CONCLUSION:

These results suggest that dokudami has anti-inflammatory effect on human gingival epithelial cells stimulated by periodontopathogens through the inhibition of inflammatory related genes.